F ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 March 2001 (13.03.01)	
International application No. PCT/CA00/00801	**Applicant's or agent's file reference** 29963-0002
International filing date (day/month/year) 06 July 2000 (06.07.00)	**Priority date** (day/month/year) 06 July 1999 (06.07.99)
Applicant VARIN, Luc et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 February 2001 (05.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

ROBIC
55 St-Jacques
Montréal, Québec H2Y 3X2
CANADA

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 11 October 2000 (11.10.00)	
Applicant's or agent's file reference 29963-0002	**IMPORTANT NOTIFICATION**
International application No. PCT/CA00/00801	International filing date (day/month/year) 06 July 2000 (06.07.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 06 July 1999 (06.07.99)
Applicant VARIN, Luc et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
06 July 1999 (06.07.99)	2,274,873	CA	09 Augu 2000 (09.08.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

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WO 01/02589
PCT/CA00/00801

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
ROBIC
55 St-Jacques
Montréal, Québec H2Y 3X2
CANADA

Date of mailing (day/month/year)
11 January 2001 (11.01.01)

Applicant's or agent's file reference
29963-0002

IMPORTANT NOTICE

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/CA00/00801	06 July 2000 (06.07.00)	06 July 1999 (06.07.99)

Applicant
VARIN, Luc et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,BZ,DZ,KP,KR,MZ,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   11 January 2001 (11.01.01) under No. WO 01/02589

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
29963-0002		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/CA00/00801	06/07/2000	06/07/1999
International Patent Classification (IPC) or national classification and IPC		
C12N15/82		
Applicant		
VARIN, Luc et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 18 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/02/2001	25.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Burkhardt, P Telephone No. +49 89 2399 7456

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00801

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-5,8-16,18-33	as originally filed	
6,7,17	with telefax of	21/09/2001

Claims, No.:

1-51	with telefax of	21/09/2001

Drawings, sheets:

1/6-6/6	as originally filed

Sequence listing part of the description, pages:

1-5, filed with the letter of 21.09.2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00801

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 35, 43 - 47
	No:	Claims	36 - 42, 48 - 51
Inventive step (IS)	Yes:	Claims	4 - 10, 12 - 18, 24 - 33, 43 - 47
	No:	Claims	1 - 3, 11, 19 - 23, 34 - 42, 48 - 51
Industrial applicability (IA)	Yes:	Claims	1 - 51
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00801

Re Item I

Basis of the opinion

The amended description and claims filed with the telefax of 21.09.2001 are formally acceptable under Article 34(2)(b) PCT.

The amendments in the sequence listing pages 1-5 filed with the telefax of 21.09.2001 appear to be corrections of an obvious error that has been detected by the ISA. The amendments are therefore formally acceptable under Article 34(2)(b) PCT under the condition that no new matter has been added.

Re Item V

Reasoned statement under Article 35 with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this report; the numbering will be adhered to in the rest of the procedure and is following the order of the International Search Report:

D1 DD-A-209379 (ADW DDR)
D2 EMBL Acc. No. AB010697
D3 Krajnčič and Nemec, 1995. J. Plant Physiol. 146:754-756.
D4 Albrechtová and Ullmann, 1994. Biol. Planta. 36:317-319.

1. **Article 33(2)(3) PCT (Novelty and inventive step)**

1.1 Present claim 1 is directed to a method of modulating flowering in a plant by modifying the endogenous level of at least one compound belonging to the jasmonate family.
Documents D1 (page 1, last paragraph), D3 (page 755, Discussion) and D4 (page 318, 2nd paragraph) disclose methods of modulating flowering in a plant by modifying the endogenous level of a compound of the jasmonate family, i.e. jasmonic or methyljasmonic acid. These two compounds are excluded from

present claim 1. Claim 1 and dependent claims 2 - 11 and 21 - 28 thus meet the requirements of Article 33(2) PCT. The same holds true for the subject-matter of claims 19, 20, 34 and 35 directed to a composition for inducing or delaying flowering in a plant comprising a compound mentioned in claim 1.

1.2 The subject-matter of present claim 1 differs from D1, D3 or D4 by the use of another compound from the jasmonate family. The problem to be solved may thus be formulated as the provision of an altemative method for modulating flowering in a plant.

1.3 Alternative compounds from the jasmonate family have been available at the filing date of the present application. It does not involve an inventive step to exchange one known compound by another known compound of the same chemical group. An inventive activity for the subject-matter of present claim 1 can therefore not be acknowledged. Claim 1 does not meet the requirements of Article 33(3) PCT. The same holds true for dependent claims 2, 3, 11 and 21 - 23 as well as for claims 19, 20,34 and 35 directed to compositions containing said compounds.

1.4 Present claim 36 is directed to an isolated nucleic acid molecule encoding a plant hydroxyjasmonic acid sulfotransferase.
Document D2 discloses a nucleic acid sequence that is 100% identical to SEQ ID NOs:1 and 3. D2 therefore anticipates the subject-matter of present claims 36 and 37 - 39 as well as of claim 48 directed to the corresponding protein. Dependent claims 40 - 42 and 49 - 51 do not contain any features that would render the subject-matter of said claims novel or inventive over the prior art presently available to the IPEA.

1.5 The function of a nucleic acid molecule is an inherent feature of its sequence. Consequently, annotating a known sequence cannot establish novelty over the prior art D2.

1.6 It appears that claims directed to a method of modulating flowering in a plant by enhancing or inhibiting the expression of AtST2a/b and thereby increasing or decreasing the endogenous level of jasmonic acid, methyljasmonic acid, 12-

hydroxyjasmonic acid and/or 11 hydroxyjasmonic (i.e. claims 4 - 10, 12 - 18, 24 - 33 and 43 - 47) acid could meet the requirements of Article 33(2)(3) PCT. Please also see the comments in section VIII below.

1.5 The applicant is requested to note that any transgenic plant containing a gene that leads to early or late flowering may anticipate the subject-matter of present claims 12, 13, 29 and 30. These plants may have, as a result of the expression of a flowering related gene (WUSCHEL, APETALA, ...), a modified level of a compound of the so-called jasmonate family.

Re Item VIII

Certain observations on the international application

1. The term "functional homologues" in present claims 6 and 31 is unclear (Article 6 PCT). It is not apparent what such a term may comprise and it is therefore not useful as a true technical feature.
   The same holds true for the term "AtST2a/b". Such internal arbitrary designations are meaningless to a man skilled in the art and should be replaced by reference to a SEQ ID NO.

2. It may be true that the description (page 8, line 27 - page 9, line 13) provides some sort of "definition" for the contested term. This "definition" is however not useful to clearly define the meaning of a "functional homologue". On the contrary, it introduces ambiguity and does not allow to determine the extent of protection.

3. Claims 12 and 29 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added. It is not apparent how an increased or decreased endogenous level of compounds of the so-called jasmonate family should be obtained.
   It furthermore appears that the subject-matter of claims 12 and 29 in their present form is not sufficiently disclosed for a man skilled in the art to carry out the

invention. The only method that has been reduced to practice is the overexpression or inhibition of AtST2a/b. Thus an undue burden is placed on others trying to establish the extent of protection (Article 5 PCT)
The same holds true for present claims 4, 5, 21 - 24 and 30.

4. Similar objections apply to present claims 20 and 35 with respect to the term "effective amount of ...". It is not apparent how an effective amount of a substance modulating flowering in a plant should be defined. Therefore, the term is not useful as a true technical feature.
The definitions provided by the description do not help to clarify the contested term. Once again an undue burden is placed on others trying to establish the extent of protection (Article 5 PCT).

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JC13 Rec'd PCT/PTO 07 JAN 2002

advantage of great economic importance for horticultural plants and some crop plants such as cauliflower and broccoli.

Other objects and advantages of the present invention will be apparent upon reading the following non-restrictive description of several preferred embodiments, made with reference to the accompanying drawings and to the enclosed examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and **1B** show the chemical structures of 12-hydroxyjasmonic acid (Fig. 1A) and 11-hydroxyjasmonic acid (Fig. 1B).

Figures 2A and **2B** are pictures showing the effect on flowering time of a treatment with 12-hydroxyjasmonic acid (Fig. 2B) in *Arabidopsis thaliana*, when compared to a treatment with water (Fig. 2A).

Figure 3 is a picture showing the phenotype of transgenic *Arabidopsis* plants expressing *AtST2a* gene under the control of a constitutive promoter when compared to wild type non-transgenic plant (WT). S5, S6, S9, and S16 indicate independent transgenic lines.

Figure 4 is a Western blot of protein extracts from the plants shown in Fig. 3 probed with anti-*AtST2a* antibodies. MW: Molecular weight markers; WT: wild type plants; S5, S6, S9, and S16: independent transgenic lines.

Figure 5 is a picture showing the phenotype of transgenic *Arabidopsis* plants expressing the *AtST2a* gene in the antisense orientation under the control of a constitutive promoter (TL 7-2-5) when compared to non transgenic plants (WT).

Figure 6 is a picture showing the effect of methyljasmonic acid treatment on the flowering time of wild type *Arabidopsis thaliana* plants (WT C24) and on transgenic *Arabidopsis thaliana* plants expressing the *AtST2a* gene in the antisense orientation under the control of a constitutive promoter (TL 7-2-5).

Figure 7: Shows nucleotide sequence of *AtST2a* gene (SEQ ID NO 1) taken from *Arabidopsis thaliana* database at Stanford University (clone number M0J9, gene MOJ9.16 and the EST 119G6T7) and the GenBank™ database (accession number AB010697, nucleotides 53936 to 55015).

Figure 8: Shows the deduced amino acid sequence (SEQ ID NO 3) of the protein encoded by the *AtST2a* gene shown in Fig. 7.

Figure 9: Shows the nucleotide sequence of *AtST2b* gene (SEQ ID NO 2) taken from *Arabidopsis thaliana* database at Stanford University (clone number M0J9, gene MOJ9.15) and the GenBank™ database (accession number AB010697, nucleotides 50627 to 51670).

Figure 10: Shows the deduced amino acid sequence (SEQ ID NO 4) of the protein encoded by the *AtST2b* gene shown in Fig. 9.

Figure 11 is a Northern blot of plants mRNA extracts showing the effect of various 12-hydroxyjasmonate concentrations on the expression of the *AtST2a* gene.

Figure 12 is a Northern blot of plants mRNA extracts showing the effect of the photoperiod on the expression of the *AtST2a* gene.

DETAILED DESCRIPTION OF THE INVENTION

A) Definitions

In order to provide an even clearer and more consistent understanding of the specification and the claims, including the scope given herein to such terms, the following definitions are provided:

11-hydroxyjasmonic acid: 3-Oxo-2-(4-hydroxy-2-pentenyl)-cyclopentane-1-acetic acid. Its chemical structure is shown in Fig. 1B.

11-hydroxyjasmonic acid glucoside: 3-Oxo-2-(4-β-D-glucopyranosyloxy-2-pentenyl)-cyclopentane-1-acetic acid

11-hydroxyjasmonic acid sulfate: 3-Oxo-2-(4-hydroxysulfonyloxy-2-pentenyl)-cyclopentane-1-acetic acid

12-hydroxyjasmonic acid: 3-Oxo-2-(5-hydroxy-2-pentenyl)-cyclopentane-1-acetic acid. Its chemical structure is shown in Fig. 1A.

12-hydroxyjasmonic acid glucoside: 3-Oxo-2-(5-β-D-glucopyranosyloxy-2-pentenyl)-cyclopentane-1-acetic acid.

12-hydroxyjasmonic acid sulfate: 3-Oxo-2-(5-hydroxysulfonyloxy-2-pentenyl)-cyclopentane-1-acetic acid.

inhibitors of jasmonic acid hydroxylase(s) should prevent the production of hydroxylated jasmonate compound(s).

As for the flowering compounds, the above stimulators and/or inhibitors can be applied in a pure form, as a mixture of a plurality of compounds or be part of a flowering delaying composition.

2) Molecular approach

In accordance with the present invention, genetic sequences encoding a plant hydroxyjasmonic acid sulfotransferase have been identified, cloned and used to generate transgenic plants.

SEQ ID NO 1 (Fig. 7; GenBank™: accession number AB010697, nucleotides 53936 to 55015; and Stanford University *Arabidopsis thaliana* database: clone number M0J9, gene MOJ9.16 and EST 119G6T7) corresponds to the gene *AtST2a* in *Arabidopsis thaliana*. SEQ ID NO 3 (Fig. 8) is an amino acid sequence deduced from SEQ ID NO 1. This amino acid sequence is of public domain and comes from the Kazusa Arabidopsis Opening Site (KAOS) of the Kazusa DNA Research Institute (KDRI) (http://www.kazusa.or.jp/kaos/; clone number M0J9, gene MOJ9.16). The present inventors have found that the *AtST2a* gene from *Arabidopsis thaliana* encodes a sulfotransferase that sulfonates 12-hydroxyjasmonic acid and 11-hydroxyjasmonic acid with high specificity. Although not shown, results obtained demonstrated that this hydroxyjasmonic acid sulfotransferase exhibits high affinity for its substrate with a Km value of 11 µM for 12-hydroxyjasmonic acid and 60 µM for 11-hydroxyjasmonic acid. The enzyme did not accept structurally related compounds such as cucurbic acid, arachidonyl alcohol or prostaglandins. Maximum enzyme activity was observed at pH 7.5 in Tris/HCl buffer and did not require divalent cations for activity. The purified recombinant protein expressed in *E. coli* migrated in SDS-PAGE at a position corresponding to approximately 35,000 daltons (see Fig. 4).

SEQ ID NO 2 (Fig. 9; GenBank™: accession number AB010697, nucleotides 50627 to 51670; and Stanford University *Arabidopsis thallana* database: clone number M0J9 gene MOJ9.15), corresponds to the gene *AtST2b* in *Arabidopsis thaliana*. SEQ ID NO 4 (Fig. 10) is an amino acid sequence deduced from SEQ ID

CLAIMS:

1. A method for modulating flowering in a plant, comprising modifying in said plant the endogenous level of at least one compound selected from the group consisting of jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, sulfate ester of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, sulfate ester of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, sulfate ester of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, sulfate ester of 11-hydroxymethyljasmonic acid, and mixtures thereof.

2. The method of claim 1, wherein flowering of said plant is induced by increasing in said plant the endogenous level of at least one flowering inducing compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, said flowering induction and said endogenous level increase being compared to a corresponding plant wherein the endogenous level of said at least one compound has not been modified.

3. The method of claim 2, wherein the endogenous level of said at least one flowering inducing compound is increased by a method selected from the group consisting of:

a) applying to said plant at least one of said flowering inducing compounds and/or salts thereof;

b) applying to said plant at least one inhibitor of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic; and

c) applying to said plant at least one stimulator of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid.

4. The method of claim 2, wherein the endogenous level of said at least one flowering inducing compound is increased by:

a) increasing in said plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) lowering in said plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

5. The method of claim 4, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic is lowered subsequently to a genetic modification of said plant.

6. The method of claim 5, wherein said genetic modification comprises the step of inhibiting the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

7. The method of claim 6, wherein said gene expression is inhibited by expressing into said plant an exogenous sequence coding for a nucleic acid sequence antisense to said gene.

8. The method of claim 7, wherein said exogenous sequence is expressed under the control of a constitutive or an inducible promoter.

9. The method of any one of claims 5 to 8, wherein said plant is transgenic.

10. The method of claim 3, wherein said plant has been genetically modified to flower early prior application thereto of said flowering compound(s), said sulfotransferase inhibitor(s) and/or said hydroxylase stimulator(s).

11. The method of any one of claims 2 to 10, wherein said plant is selected from crop plants.

12. A plant genetically modified to flower early when compared to a corresponding plant not genetically modified, said genetically modified plant having an increased endogenous level of jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, when compared to said corresponding non-genetically modified plant.

13. The plant of claim 12, wherein said genetic modification comprises:

a) increasing in said genetically modified plant the endogenous level an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) lowering in said genetically modified plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

14. The genetically modified plant of claim 12 or 13, wherein said genetic modification comprises inhibiting the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

15. The genetically modified plant of claim 13, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-

hydroxyjasmonic is lowered by expressing into said plant an exogenous nucleic acid sequence, said exogenous nucleic acid sequence encoding: i) for a nucleic acid sequence antisense to a gene encoding at least one of said sulfotransferases; or ii) for a nucleic acid sequence antisense to a portion of said gene.

16. The genetically modified plant of claim 15, wherein said exogenous sequence is expressed under the control of a constitutive or inducible promoter.

17. The genetically modified plant of any one of claims 12 to 16, wherein said plant is transgenic.

18. A cut flower from the genetically modified plant of any one of claims 12 to 17.

19. A composition for inducing flowering in a plant comprising a flowering inducing effective amount of a compound selected from the group consisting of, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, salts thereof, and mixtures thereof, in combination with a diluent or a carrier such that an induction in flowering of said plant occurs when compared to a corresponding plant in the absence of said composition.

20. The composition of claim 19, further comprising a compound selected from the group consisting of fertilizers, growth regulators, fungicides, insecticides, emulsifying agents and mixtures thereof.

21. The method of claim 1, wherein flowering of said plant is delayed by lowering in said plant the endogenous level of at least one compound selected from the group consisting of jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 12-hydroxymethyljasmonic acid, said flowering delay and said lower endogenous level being compared to a corresponding plant wherein the endogenous level of said at least one compound has not been modified.

22. The method of claim 21, wherein the endogenous level of said at least one compound is lowered by:

a) applying to said plant an inhibitor and/or an inactivator of at least one of said compounds;

b) applying to said plant at least one stimulator of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic; and/or

c) applying to said plant at least one inhibitor of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid.

23. The method of claim 21, wherein the endogenous level of said at least one compound is lowered by:

a) lowering in said plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) increasing in said plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic acid.

24. The method of claim 23, wherein the endogenous level of said sulfotransferase is increased subsequently to a genetic modification in the genome of said plant.

25. The method of claim 24, wherein said genetic modification comprises the steps of increasing the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

26. The method of claim 25, wherein said gene expression is increased by placing said gene under the control of a constitutive or of an inducible promoter.

27. The method of any one of claims 21 to 26, wherein said plant is transgenic.

28. The method of claim 22, wherein said plant has been genetically modified to flower lately prior application thereto of said compound(s), said sulfotransferase stimulator(s) and/or said hydroxylase inhibitor(s).

29. A plant genetically modified to flower tardily when compared to a corresponding plant not genetically modified, said genetically modified plant having a lowered endogenous level of at least one compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, when compared to said corresponding non-genetically modified plant.

30. The plant of claim 29, wherein said genetic modification comprises:

a) lowering in said genetically modified plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) increasing in said genetically modified plant the endogenous level a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

31. The genetically modified plant of claims 29 or 30, wherein said genetic modification comprises increasing the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or *AtST2b*.

32. The genetically modified plant of claim 30, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic is increased by expressing into said genetically modified plant a nucleic acid sequence encoding said sulfotransferase under the control of a constitutive or an inducible promoter.

33. The genetically modified plant of any one of claims 29 to 32, wherein said plant is transgenic.

34. A composition for delaying flowering in a plant comprising a flowering delaying effective amount of an inhibitor or of an inactivator of a compound selected from the group consisting of jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, in combination with a diluent or a carrier such that a delay in flowering of said plant

occurs when compared to a corresponding plant in the absence of said composition.

35. The composition of claim 34, further comprising a compound selected from the group consisting of fertilizers, growth regulators, fungicides, insecticides, emulsifying agents and mixtures thereof.

36. An isolated or purified nucleic acid molecule encoding a plant 11-hydroxyjasmonic acid or 12-hydroxyjasmonic acid sulfotransferase.

37. The isolated nucleic acid molecule of claim 36, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2, nucleotide sequences having at least 50% similarity with SEQ ID NO:2 and nucleotide sequences complementary thereto.

38. The isolated nucleic acid molecule of claim 36, comprising a nucleotide sequence which
hybridizes under low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, a complementary strand of SEQ ID NO:1, SEQ ID NO:2 and a complementary strand of SEQ ID NO:2.

39. The isolated nucleic acid molecule of any one of claims 36 to 38, wherein the hydroxyjasmonic acid sulfotransferase is of *Arabidopsis thaliana* origin.

40. A vector comprising the nucleic acid molecule of any one of claims 36 to 39.

41. The vector of claim 40, wherein the vector is capable of replication and expression in a plant cell.

42. A transgenic plant comprising the nucleic acid molecule of any one of claims 36 to 39.

43. A method for producing a transgenic plant capable to flower early, said method comprising the steps of:

a) introducing into a cell of a suitable plant an exogenous nucleic acid molecule comprising a sequence of nucleotides antisense to a sequence encoding a plant hydroxyjasmonic acid sulfotransferase;

b) regenerating a transgenic plant from the cell; and

c) growing said transgenic plant for a time and under conditions sufficient to inhibit expression of the hydroxyjasmonic acid sulfotransferase.

44. The method of claim 43, wherein the exogenous nucleic acid molecule comprises a nucleotide sequence antisense to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2 and nucleotide sequences having at least 50% similarity with SEQ ID NO:2.

45. A method for producing a transgenic plant capable to flower tardily, said method comprising the steps of:

a) introducing into a cell of a suitable plant an exogenous nucleic acid molecule encoding a plant hydroxyjasmonic acid sulfotransferase;

b) regenerating a transgenic plant from the cell; and

c) growing said transgenic plant for a time and under conditions sufficient to permit expression of the nucleic acid sequence into an hydroxyjasmonic acid sulfotransferase.

46. The method of claim 45, wherein the exogenous nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2 and nucleotide sequences having at least 50% similarity with SEQ ID NO:2.

47. The method of any one of claims 43 to 46, wherein the hydroxyjasmonic acid sulfotransferase is a 11- or a 12- hydroxyjasmonic acid sulfotransferase.

48. An isolated or purified polypeptide having the biological activity of a plant 11-hydroxyjasmonic acid or 12-hydroxyjasmonic acid sulfotransferase.

49. The polypeptide of claim 48, encoding a sulfotransferase enzyme selected from the group consisting of:

a) an enzyme whose amino acid sequence is represented by SEQ ID NO: 3 or SEQ ID NO: 4; and

b) functional homologues of enzyme a) isolated from a plant, or derived from enzyme a) by substitution, deletion or addition of one or several amino acids in the amino acid sequences defined in a) and having similar biological activity or function(s).

50. An antibody binding with affinity to a polypeptide as defined in claim 48 or 49.

51. The antibody of claim 50 used for delaying flowering in a plant.

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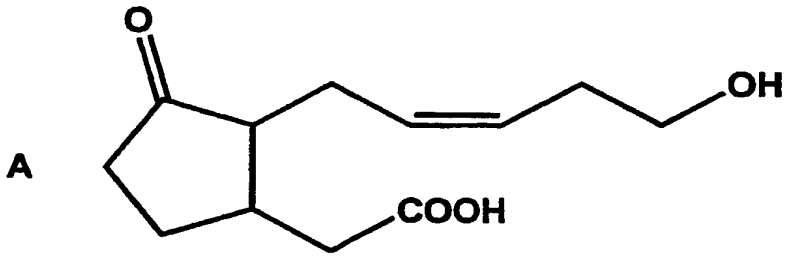
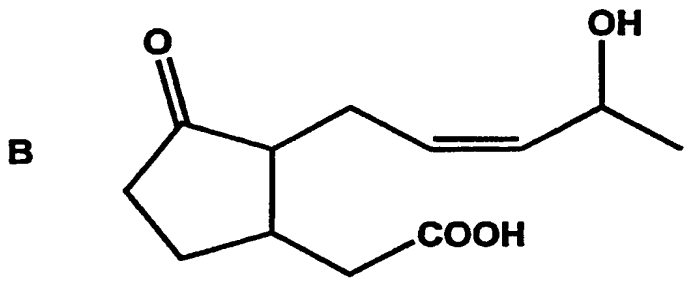
(54) Title: METHODS, COMPOSITIONS AND GENETIC SEQUENCES FOR MODULATING FLOWERING IN PLANTS, AND PLANTS GENETICALLY MODIFIED TO FLOWER EARLY AND TARDILY





(57) Abstract: The present invention relates to methods, compositions and genetic sequences for modulating flowering in plants and to plants genetically modified to flower early and to plants genetically modified to flower tardily. More particularly the present invention provides among others a genetic sequence encoding for a hydroxyjasmonic acid sulfotransferase and methods for producing transgenic plants using such a sequence.

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INTERNATIONAL SEARCH REPORT

Inte[redacted] Application No
PCT/[redacted]00/00801

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/54 C12N9/10 C07K16/40 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DD 209 379 A (ADW DDR) 9 May 1984 (1984-05-09) the whole document ---	1-4,12, 20,21
X	DATABASE EMBL SEQUENCE DATABASE 'Online! 3 February 1998 (1998-02-03) KANEKO, T., ET AL. : "structural analysis of Arabidopsis thaliana chromosome 5. V. Sequence features of the regions of 1,381,565 bp covered by twenty one physically assigned P1 and TAC clones" XP002161012 accession no. AB010697 --- -/--	37-41, 50,51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

Inte[redacted] Application No
PCT/[redacted]00/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KRAJNCIC BOZIDAR ET AL: "The effect of jasmonic-acid on flowering in Spirodela polyrrhiza (L.) Schleiden." JOURNAL OF PLANT PHYSIOLOGY, vol. 146, no. 5-6, 1995, pages 754-756, XP000986869 ISSN: 0176-1617 the whole document	1-4
X	ALBRECHTOVA J T P ET AL: "Methyl jasmonate inhibits growth and flowering in Chenopodium rubrum." BIOLOGIA PLANTARUM (PRAGUE), vol. 36, no. 2, 1994, pages 317-319, XP000986811 ISSN: 0006-3134 the whole document	1
A	FEYS B J F ET AL: "ARABIDOPSIS MUTANTS SELECTED FOR RESISTANCE TO THE PHYTOTOXIN CORONATINE ARE MALE STERILE, INSENSITIVE TO METHYL JASMONATE AND RESISTANT TO A BACTERIAL PATHOGEN" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 6, 1 May 1994 (1994-05-01), pages 751-759, XP002049621 ISSN: 1040-4651 page 756	
A	EP 0 777 965 A (TAMA BIOCHEMICAL CO LTD ;NIPPON ZEON CO (JP)) 11 June 1997 (1997-06-11) page 9, line 41 - line 42	
E	EP 1 033 405 A (CERES INC) 6 September 2000 (2000-09-06) see SEQID 558964 abstract	37-44, 50,51

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The SEQID2 is not representing a nucleotide sequence but indeed is representing an amino acid sequence according to the filed sequence listings; SEQID3 is representing a nucleotide sequence according to said sequence listings.
Accordingly, the search was based on the assumption that the nucleotide sequences of claims 38,39,46 and 48 are represented by SEQIDs 1 and 3 and the amino acid sequences of claim 50 are represented by SEQIDs 2 and 4, respectively.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

Inte[redacted] Application No
PCT/[redacted] 00/00801

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DD 209379 A	09-05-1984	CU 35622 A	05-05-1983
EP 0777965 A	11-06-1997	AU 3265495 A	22-03-1996
		BR 9508636 A	25-11-1997
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		CN 1168619 A	24-12-1997
		WO 9606529 A	07-03-1996
		PL 318809 A	07-07-1997
EP 1033405 A	06-09-2000	NONE	

advantage of great economic importance for horticultural plants and some crop plants such as cauliflower and broccoli.

Other objects and advantages of the present invention will be apparent upon reading the following non-restrictive description of several preferred embodiments, made with reference to the accompanying drawings and to the enclosed examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and **1B** show the chemical structures of 12-hydroxyjasmonic acid (Fig. 1A) and 11-hydroxyjasmonic acid (Fig. 1B).

Figures 2A and **2B** are pictures showing the effect on flowering time of a treatment with 12-hydroxyjasmonic acid (Fig. 2B) in *Arabidopsis thaliana,* when compared to a treatment with water (Fig. 2A).

Figure 3 is a picture showing the phenotype of transgenic *Arabidopsis* plants expressing *AtST2a* gene under the control of a constitutive promoter when compared to wild type non-transgenic plant (WT). S5, S6, S9, and S16 indicate independent transgenic lines.

Figure 4 is a Western blot of protein extracts from the plants shown in Fig. 3 probed with anti-*AtST2a* antibodies. MW: Molecular weight markers; WT: wild type plants; S5, S6, S9, and S16: independent transgenic lines.

Figure 5 is a picture showing the phenotype of transgenic *Arabidopsis* plants expressing the *AtST2a* gene in the antisense orientation under the control of a constitutive promoter (TL 7-2-5) when compared to non transgenic plants (WT).

Figure 6 is a picture showing the effect of methyljasmonic acid treatment on the flowering time of wild type *Arabidopsis thaliana* plants (WT C24) and on transgenic *Arabidopsis thaliana* plants expressing the *AtST2a* gene in the antisense orientation under the control of a constitutive promoter (TL 7-2-5).

Figure 7: Shows nucleotide sequence of *AtST2a* gene (SEQ ID NO 1) taken from *Arabidopsis thaliana* database at Stanford University (clone number M0J9, gene MOJ9.16 and the EST 119G6T7) and the GeneBank database (accession number AB010697, nucleotides 55015 to 53936).

Figure 8: Shows the deduced amino acid sequence (SEQ ID NO 3) of the protein encoded by the *AtST2a* gene shown in Fig. 7.

Figure 9: Shows the nucleotide sequence of *AtST2b* gene (SEQ ID NO 2) taken from *Arabidopsis thaliana* database at Stanford University (clone number M0J9, gene MOJ9.15) and the GeneBank database (accession number AB010697, nucleotides 51670 to 50627).

Figure 10: Shows the deduced amino acid sequence (SEQ ID NO 4) of the protein encoded by the *AtST2b* gene shown in Fig. 9.

Figure 11 is a Northern blot of plants mRNA extracts showing the effect of various 12-hydroxyjasmonate concentrations on the expression of the *AtST2a* gene.

Figure 12 is a Northern blot of plants mRNA extracts showing the effect of the photoperiod on the expression of the *AtST2a* gene.

DETAILED DESCRIPTION OF THE INVENTION

A) Definitions

In order to provide an even clearer and more consistent understanding of the specification and the claims, including the scope given herein to such terms, the following definitions are provided:

11-hydroxyjasmonic acid: 3-Oxo-2-(4-hydroxy-2-pentenyl)-cyclopentane-1-acetic acid. Its chemical structure is shown in Fig. 1B.

11-hydroxyjasmonic acid glucoside: 3-Oxo-2-(4-β-D-glucopyranosyloxy-2-pentenyl)-cyclopentane-1-acetic acid

11-hydroxyjasmonic acid sulfate: 3-Oxo-2-(4-hydroxysulfonyloxy-2-pentenyl)-cyclopentane-1-acetic acid

12-hydroxyjasmonic acid: 3-Oxo-2-(5-hydroxy-2-pentenyl)-cyclopentane-1-acetic acid. Its chemical structure is shown in Fig. 1A.

12-hydroxyjasmonic acid glucoside: 3-Oxo-2-(5-β-D-glucopyranosyloxy-2-pentenyl)-cyclopentane-1-acetic acid.

12-hydroxyjasmonic acid sulfate: 3-Oxo-2-(5-hydroxysulfonyloxy-2-pentenyl)-cyclopentane-1-acetic acid.

inhibitors of jasmonic acid hydroxylase(s) should prevent the production of hydroxylated jasmonate compound(s).

As for the flowering compounds, the above stimulators and/or inhibitors can be applied in a pure form, as a mixture of a plurality of compounds or be part of a flowering delaying composition.

2) Molecular approach

In accordance with the present invention, genetic sequences encoding a plant hydroxyjasmonic acid sulfotransferase have been identified, cloned and used to generate transgenic plants.

SEQ ID NO 1 (Fig. 7; GeneBank: accession number AB010697, nucleotides 53939 to 55015; and Stanford University *Arabidopsis thaliana* database: clone number M0J9, gene MOJ9.16 and EST 119G6T7) corresponds to the gene *AtST2a* in *Arabidopsis thaliana*. SEQ ID NO 3 (Fig. 8) is an amino acid sequence deduced from SEQ ID NO 1. This amino acid sequence is of public domain and comes from the Kazusa Arabidopsis Opening Site (KAOS) of the Kazusa DNA Research Institute (KDRI) (http://www.kazusa.or.jp/kaos/; clone number M0J9, gene MOJ9.16). The present inventors have found that the *AtST2a* gene from *Arabidopsis thaliana* encodes a sulfotransferase that sulfonates 12-hydroxyjasmonic acid and 11-hydroxyjasmonic acid with high specificity. Although not shown, results obtained demonstrated that this hydroxyjasmonic acid sulfotransferase exhibits high affinity for its substrate with a Km value of 11 µM for 12-hydroxyjasmonic acid and 60 µM for 11-hydroxyjasmonic acid. The enzyme did not accept structurally related compounds such as cucurbic acid, arachidonyl alcohol or prostaglandins. Maximum enzyme activity was observed at pH 7.5 in Tris/HCl buffer and did not require divalent cations for activity. The purified recombinant protein expressed in *E. coli* migrated in SDS-PAGE at a position corresponding to approximately 35,000 daltons (see Fig. 4).

SEQ ID NO 2 (Fig. 9; GeneBank: accession number AB010697, nucleotides 50630 to 51670; and Stanford University *Arabidopsis thaliana* database: clone number M0J9 gene MOJ9.15), corresponds to the gene *AtST2b* in *Arabidopsis thaliana*. SEQ ID NO 4 (Fig. 10) is an amino acid sequence deduced from SEQ ID

CLAIMS:

1. A method for modulating flowering in a plant, comprising modifying in said plant the endogenous level of at least one compound of the jasmonate family.

2. The method of claim 1, wherein said compound is selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, sulfate ester of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, sulfate ester of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, sulfate ester of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, sulfate ester of 11-hydroxymethyljasmonic acid, and mixtures thereof.

3. The method of claim 1 or 2, wherein flowering of said plant is induced by increasing in said plant the endogenous level of at least one flowering inducing compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, said flowering induction and said endogenous level increase being compared to a corresponding plant wherein the endogenous level of said at least one compound has not been modified.

4. The method of claim 3, wherein the endogenous level of said at least one flowering inducing compound is increased by a method selected from the group consisting of:

a) applying to said plant at least one of said flowering inducing compounds and/or salts therof;

b) applying to said plant at least one inhibitor of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic; and

c) applying to said plant at least one stimulator of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid.

5. The method of claim 3, wherein the endogenous level of said at least one flowering inducing compound is increased by:

a) increasing in said plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) lowering in said plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

6. The method of claim 5, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic is lowered subsequently to a genetic modification of said plant.

7. The method of claim 6, wherein said genetic modification comprises the step of inhibiting the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

8. The method of claim 7, wherein said gene expression is inhibited by expressing into said plant an exogenous sequence coding for a nucleic acid sequence antisense to said gene.

9. The method of claim 8, wherein said exogenous sequence is expressed under the control of a constitutive or an inducible promoter.

10. The method of any one of claims 6 to 9, wherein said plant is transgenic.

11. The method of claim 4, wherein said plant has been genetically modified to flower early prior application thereto of said flowering compound(s), said sulfotransferase inhibitor(s) and/or said hydroxylase stimulator(s).

12. The method of any one of claims 3 to 11, wherein said plant is selected from crop plants.

13. A plant genetically modified to flower early when compared to a corresponding plant not genetically modified, said genetically modified plant having an increased endogenous level of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, when compared to said corresponding non-genetically modified plant.

14. The plant of claim 13, wherein said genetic modification comprises:

a) increasing in said genetically modified plant the endogenous level an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) lowering in said genetically modified plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

15. The genetically modified plant of claim 13 or 14, wherein said genetic modification comprises inhibiting the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

16. The genetically modified plant of claim 14, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic is lowered by expressing into said plant an exogenous nucleic acid sequence, said exogenous nucleic acid sequence encoding: i) for a nucleic acid sequence antisense to a gene encoding at least one of said sulfotransferases; or ii) for a nucleic acid sequence antisense to a portion of said gene.

17. The genetically modified plant of claim 16, wherein said exogenous sequence is expressed under the control of a constitutive or inducible promoter.

18. The genetically modified plant of any one of claims 13 to 17, wherein said plant is transgenic.

19. A cut flower from the genetically modified plant of any one of claims 13 to 18.

20. A composition for inducing flowering in a plant comprising a flowering inducing effective amount of a compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, salts thereof, and mixtures thereof, in combination with a diluent or a carrier such that an induction in flowering of said plant occurs when compared to a corresponding plant in the absence of said composition.

21. The composition of claim 20, further comprising a compound selected from the group consisting of fertilizers, growth regulators, fungicides, insecticides, emulsifying agents and mixtures thereof.

22. The method of claim 1 or 2, wherein flowering of said plant is delayed by lowering in said plant the endogenous level of at least one compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 12-hydroxymethyljasmonic acid, said flowering delay and said lower endogenous level being compared to a corresponding plant wherein the endogenous level of said at least one compound has not been modified.

23. The method of claim 22, wherein the endogenous level of said at least one compound is lowered by:

a) applying to said plant an inhibitor and/or an inactivator of at least one of said compounds;

b) applying to said plant at least one stimulator of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic; and/or

c) applying to said plant at least one inhibitor of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid.

24. The method of claim 22, wherein the endogenous level of said at least one compound is lowered by:

a) lowering in said plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) increasing in said plant the endogenous level of a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic acid.

25. The method of claim 24, wherein the endogenous level of said sulfotransferase is increased subsequently to a genetic modification in the genome of said plant.

26. The method of claim 25, wherein said genetic modification comprises the steps of increasing the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or of *AtST2b*.

27. The method of claim 26, wherein said gene expression is increased by placing said gene under the control of a constitutive or of an inducible promoter.

28. The method of any one of claims 22 to 27, wherein said plant is transgenic.

29. The method of claim 23, wherein said plant has been genetically modified to flower lately prior application thereto of said compound(s), said sulfotransferase stimulator(s) and/or said hydroxylase inhibitor(s).

30. A plant genetically modified to flower tardily when compared to a corresponding plant not genetically modified, said genetically modified plant having a lowered endogenous level of at least one compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-

hydroxymethyljasmonic acid, when compared to said corresponding non-genetically modified plant.

31. The plant of claim 31, wherein said genetic modification comprises:

a) lowering in said genetically modified plant the endogenous level of an hydroxylase hydroxylating jasmonic acid and/or methyljasmonic acid; and/or

b) increasing in said genetically modified plant the endogenous level a sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic.

32. The genetically modified plant of claims 30 or 31, wherein said genetic modification comprises increasing the expression of at least one gene selected from the group consisting of *AtST2a*, *AtST2b* and functional homologues of *AtST2a* or *AtST2b*.

33. The genetically modified plant of claim 31, wherein the endogenous level of the sulfotransferase sulfonating 12-hydroxyjasmonic acid and/or 11-hydroxyjasmonic is increased by expressing into said genetically modified plant a nucleic acid sequence encoding said sulfotransferase under the control of a constitutive or an inducible promoter.

34. The genetically modified plant of any one of claims 30 to 33, wherein said plant is transgenic.

35. A composition for delaying flowering in a plant comprising a flowering delaying effective amount of an inhibitor or of an inactivator of a compound selected from the group consisting of jasmonic acid, jasmonic acid-tyrosine conjugate, jasmonic acid-tryptophan conjugate, jasmonic acid-phenylalanine conjugate, jasmonic acid-isoleucine conjugate, jasmonic acid-leucine conjugate, jasmonic acid-valine conjugate, 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, methyljasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, 11-hydroxyjasmonic acid, glucoside

of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, and glucoside of 11-hydroxymethyljasmonic acid, in combination with a diluent or a carrier such that a delay in flowering of said plant occurs when compared to a corresponding plant in the absence of said composition.

36. The composition of claim 35, further comprising a compound selected from the group consisting of fertilizers, growth regulators, fungicides, insecticides, emulsifying agents and mixtures thereof.

37. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a plant hydroxyjasmonic acid sulfotransferase.

38. The isolated nucleic acid molecule of claim 37, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2, nucleotide sequences having at least 50% similarity with SEQ ID NO:2 and nucleotide sequences complementary thereto.

39. An isolated nucleic acid molecule which:

i) encodes an hydroxyjasmonic acid sulfotransferase of plant origin; and

ii) hybridizes under low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, a complementary strand of SEQ ID NO:1, SEQ ID NO:2 and a complementary strand of SEQ ID NO:2.

40. The isolated nucleic acid molecule of any one of claims 37 to 39, wherein the hydroxyjasmonic acid sulfotransferase is of *Arabidopsis thaliana* origin.

41. The isolated nucleic acid molecule of any one of claims 37 to 40, wherein the hydroxyjasmonic acid sulfotransferase is a 11- or a 12- hydroxyjasmonic acid sulfotransferase.

42. A vector comprising the nucleic acid molecule of any one of claims 37 to 41.

43. The vector of claim 42, wherein the vector is capable of replication and expression in a plant cell.

44. A transgenic plant comprising the nucleic acid molecule of any one of claims 37 to 41.

45. A method for producing a transgenic plant capable to flower early, said method comprising the steps of:

a) introducing into a cell of a suitable plant an exogenous nucleic acid molecule comprising a sequence of nucleotides antisense to a sequence encoding a plant hydroxyjasmonic acid sulfotransferase;

b) regenerating a transgenic plant from the cell; and

c) growing said transgenic plant for a time and under conditions sufficient to inhibit expression of the hydroxyjasmonic acid sulfotransferase.

46. The method of claim 45, wherein the exogenous nucleic acid molecule comprises a nucleotide sequence antisense to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2 and nucleotide sequences having at least 50% similarity with SEQ ID NO:2.

47. A method for producing a transgenic plant capable to flower tardily, said method comprising the steps of:

a) introducing into a cell of a suitable plant an exogenous nucleic acid molecule encoding a plant hydroxyjasmonic acid sulfotransferase;

b) regenerating a transgenic plant from the cell; and

c) growing said transgenic plant for a time and under conditions sufficient to permit expression of the nucleic acid sequence into an hydroxyjasmonic acid sulfotransferase.

48. The method of claim 47, wherein the exogenous nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, nucleotide sequences having at least 50% similarity with SEQ ID NO:1, SEQ ID NO:2 and nucleotide sequences having at least 50% similarity with SEQ ID NO:2.

49. The method of any one of claims 45 to 48, wherein the hydroxyjasmonic acid sulfotransferase is a 11- or a 12- hydroxyjasmonic acid sulfotransferase.

50. An isolated hydroxyjasmonic acid sulfotransferase enzyme selected from the group of:

a) an enzyme whose amino acid sequence is represented by SEQ ID NO 3 or SEQ ID NO 4; and

b) functional homologues of enzyme a) isolated from a plant, or derived from enzyme a) by substitution, deletion or addition of one or several amino acids in the amino acid sequences defined in a) and having similar biological activity or function(s).

51. An antibody binding with affinity to at least one of the hydroxyjasmonic acid sulfotransferase of claim 50.

52. The antibody of claim 51 used for delaying flowering in a plant.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
29963-0002		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00801	06/07/2000	06/07/1999
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant VARIN, Luc et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 18 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/02/2001	25.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Burkhardt, P Telephone No. +49 89 2399 7456

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00801

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-5,8-16,18-33	as originally filed	
6,7,17	with telefax of	21/09/2001

Claims, No.:

1-51	with telefax of	21/09/2001

Drawings, sheets:

1/6-6/6	as originally filed

Sequence listing part of the description, pages:

1-5, filed with the letter of 21.09.2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 35, 43 - 47
	No:	Claims	36 - 42, 48 - 51
Inventive step (IS)	Yes:	Claims	4 - 10, 12 - 18, 24 - 33, 43 - 47
	No:	Claims	1 - 3, 11, 19 - 23, 34 - 42, 48 - 51
Industrial applicability (IA)	Yes:	Claims	1 - 51
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the opinion

The amended description and claims filed with the telefax of 21.09.2001 are formally acceptable under Article 34(2)(b) PCT.

The amendments in the sequence listing pages 1-5 filed with the telefax of 21.09.2001 appear to be corrections of an obvious error that has been detected by the ISA. The amendments are therefore formally acceptable under Article 34(2)(b) PCT under the condition that no new matter has been added.

Re Item V

Reasoned statement under Article 35 with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this report; the numbering will be adhered to in the rest of the procedure and is following the order of the International Search Report:

D1 DD-A-209379 (ADW DDR)
D2 EMBL Acc. No. AB010697
D3 Krajnčič and Nemec, 1995. J. Plant Physiol. 146:754-756.
D4 Albrechtová and Ullmann, 1994. Biol. Planta. 36:317-319.

1. Article 33(2)(3) PCT (Novelty and inventive step)

1.1 Present claim 1 is directed to a method of modulating flowering in a plant by modifying the endogenous level of at least one compound belonging to the jasmonate family.
Documents D1 (page 1, last paragraph), D3 (page 755, Discussion) and D4 (page 318, 2nd paragraph) disclose methods of modulating flowering in a plant by modifying the endogenous level of a compound of the jasmonate family, i.e. jasmonic or methyljasmonic acid. These two compounds are excluded from

present claim 1. Claim 1 and dependent claims 2 - 11 and 21 - 28 thus meet the requirements of Article 33(2) PCT. The same holds true for the subject-matter of claims 19, 20, 34 and 35 directed to a composition for inducing or delaying flowering in a plant comprising a compound mentioned in claim 1.

1.2 The subject-matter of present claim 1 differs from D1, D3 or D4 by the use of another compound from the jasmonate family. The problem to be solved may thus be formulated as the provision of an alternative method for modulating flowering in a plant.

1.3 Alternative compounds from the jasmonate family have been available at the filing date of the present application. It does not involve an inventive step to exchange one known compound by another known compound of the same chemical group. An inventive activity for the subject-matter of present claim 1 can therefore not be acknowledged. Claim 1 does not meet the requirements of Article 33(3) PCT. The same holds true for dependent claims 2, 3, 11 and 21 - 23 as well as for claims 19, 20,34 and 35 directed to compositions containing said compounds.

1.4 Present claim 36 is directed to an isolated nucleic acid molecule encoding a plant hydroxyjasmonic acid sulfotransferase.
Document D2 discloses a nucleic acid sequence that is 100% identical to SEQ ID NOs:1 and 3. D2 therefore anticipates the subject-matter of present claims 36 and 37 - 39 as well as of claim 48 directed to the corresponding protein. Dependent claims 40 - 42 and 49 - 51 do not contain any features that would render the subject-matter of said claims novel or inventive over the prior art presently available to the IPEA.

1.5 The function of a nucleic acid molecule is an inherent feature of its sequence. Consequently, annotating a known sequence cannot establish novelty over the prior art D2.

1.4 It appears that claims directed to a method of modulating flowering in a plant by enhancing or inhibiting the expression of AtST2a/b and thereby increasing or decreasing the endogenous level of jasmonic acid, methyljasmonic acid, 12-

hydroxyjasmonic acid and/or 11 hydroxyjasmonic (i.e. claims 4 - 10, 12 - 18, 24 - 33 and 43 - 47) acid could meet the requirements of Article 33(2)(3) PCT. Please also see the comments in section VIII below.

1.5 The applicant is requested to note that any transgenic plant containing a gene that leads to early or late flowering may anticipate the subject-matter of present claims 12, 13, 29 and 30. These plants may have, as a result of the expression of a flowering related gene (WUSCHEL, APETALA, ...), a modified level of a compound of the so-called jasmonate family.

Re Item VIII

Certain observations on the international application

1. The term "functional homologues" in present claims 6 and 31 is unclear (Article 6 PCT). It is not apparent what such a term may comprise and it is therefore not useful as a true technical feature.
   The same holds true for the term "AtST2a/b". Such internal arbitrary designations are meaningless to a man skilled in the art and should be replaced by reference to a SEQ ID NO.

2. It may be true that the description (page 8, line 27 - page 9, line 13) provides some sort of "definition" for the contested term. This "definition" is however not useful to clearly define the meaning of a "functional homologue". On the contrary, it introduces ambiguity and does not allow to determine the extent of protection.

3. Claims 12 and 29 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added. It is not apparent how an increased or decreased endogenous level of compounds of the so-called jasmonate family should be obtained.
   It furthermore appears that the subject-matter of claims 12 and 29 in their present form is not sufficiently disclosed for a man skilled in the art to carry out the

invention. The only method that has been reduced to practice is the overexpression or inhibition of AtST2a/b. Thus an undue burden is placed on others trying to establish the extent of protection (Article 5 PCT)
The same holds true for present claims 4, 5, 21 - 24 and 30.

4. Similar objections apply to present claims 20 and 35 with respect to the term "effective amount of ...". It is not apparent how an effective amount of a substance modulating flowering in a plant should be defined. Therefore, the term is not useful as a true technical feature.
The definitions provided by the description do not help to clarify the contested term. Once again an undue burden is placed on others trying to establish the extent of protection (Article 5 PCT).

ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
29963-0002		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00801	06/07/2000	06/07/1999
Applicant		
VARIN, Luc		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

METHODS, COMPOSITIONS AND GENETIC SEQUENCES FOR MODULATING FLOWERING IN PLANTS, AND PLANTS GENETICALLY MODIFIED TO FLOWER FARLY AND TARDILY

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The SEQID2 is not representing a nucleotide sequence but indeed is representing an amino acid sequence according to the filed sequence listings; SEQID3 is representing a nucleotide sequence according to said sequence listings.
Accordingly, the search was based on the assumption that the nucleotide sequences of claims 38,39,46 and 48 are represented by SEQIDs 1 and 3 and the amino acid sequences of claim 50 are represented by SEQIDs 2 and 4, respectively.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]CA 00/00801

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/54 C12N9/10 C07K16/40 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DD 209 379 A (ADW DDR) 9 May 1984 (1984-05-09) the whole document ---	1-4,12, 20,21
X	DATABASE EMBL SEQUENCE DATABASE 'Online! 3 February 1998 (1998-02-03) KANEKO, T., ET AL. : "structural analysis of Arabidopsis thaliana chromosome 5. V. Sequence features of the regions of 1,381,565 bp covered by twenty one physically assigned P1 and TAC clones" XP002161012 accession no. AB010697 --- -/--	37-41, 50,51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2001	06/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KRAJNCIC BOZIDAR ET AL: "The effect of jasmonic-acid on flowering in Spirodela polyrrhiza (L.) Schleiden." JOURNAL OF PLANT PHYSIOLOGY, vol. 146, no. 5-6, 1995, pages 754-756, XP000986869 ISSN: 0176-1617 the whole document	1-4
X	ALBRECHTOVA J T P ET AL: "Methyl jasmonate inhibits growth and flowering in Chenopodium rubrum." BIOLOGIA PLANTARUM (PRAGUE), vol. 36, no. 2, 1994, pages 317-319, XP000986811 ISSN: 0006-3134 the whole document	1
A	FEYS B J F ET AL: "ARABIDOPSIS MUTANTS SELECTED FOR RESISTANCE TO THE PHYTOTOXIN CORONATINE ARE MALE STERILE, INSENSITIVE TO METHYL JASMONATE AND RESISTANT TO A BACTERIAL PATHOGEN" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 6, 1 May 1994 (1994-05-01), pages 751-759, XP002049621 ISSN: 1040-4651 page 756	
A	EP 0 777 965 A (TAMA BIOCHEMICAL CO LTD ;NIPPON ZEON CO (JP)) 11 June 1997 (1997-06-11) page 9, line 41 - line 42	
E	EP 1 033 405 A (CERES INC) 6 September 2000 (2000-09-06) see SEQID 558964 abstract	37-44, 50,51

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/CA 00/00801

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DD 209379 A	09-05-1984	CU 35622 A	05-05-1983
EP 0777965 A	11-06-1997	AU 3265495 A	22-03-1996
		BR 9508636 A	25-11-1997
		US 5814581 A	29-09-1998
		CA 2198481 A	07-03-1996
		CN 1168619 A	24-12-1997
		WO 9606529 A	07-03-1996
		PL 318809 A	07-07-1997
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